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Plaur and *Plat* genes are early upregulated in response to environmental novelty in mouse brain

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Abstract

Understanding the molecular underpinnings of learning and memory processes remains a focal point in neuroscience. Exposure to novel environments promotes learning and memory formation. This study investigated the role of two activity-regulated immediate early genes, *Plaur* (encodes urokinase receptor, uPAR) and *Plat* (encodes tissue plasminogen activator, tPA), in response to environmental novelty in the mouse brain. As integral components of the plasminogen activator system, these genes contribute to synaptic plasticity, neuronal migration, and brain function. Using the open field as a model of novelty, we demonstrated a rapid, within 1 h after exposure, induction of *Plaur* and *Plat* expression in the posterior cortex and hippocampus. Immunofluorescence staining corroborates the upregulation of tPA protein in hippocampus 24 h following open field exposure. Additionally, a brief one-hour exposure to an enriched environment triggers an early induction of *Plaur* expression in the anterior cortex, while prolonged exposure for 24 h results in a transient downregulation of *Plat* in the posterior cortex. These findings highlight the dynamic regulation of immediate early genes in response to environmental novelty, providing insights into the molecular mechanisms underlying cognitive processes and the involvement of the plasminogen activator system in these processes. Further analysis of the expression of plasminogen activator genes under conditions of novelty exposure and learning will allow us to identify new molecular targets that describe the mechanisms of learning and memory encoding in the brain.

Keywords *Plaur* · *Plat* · Immediate early genes · UPAR · TPA

Abbreviations

Arc	Activity-regulated cytoskeleton-associated protein
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
EE	Enriched environment
ERK	Extracellular regulated kinase

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NCAM	Neuronal cell adhesion molecule
NMDA	Glutamate receptor (N-methyl-D-aspartate)
PBS	Phosphate-buffered saline
PCR	Polymerase chain reactio
PKMζ	Plasticity-related protein kinase Mζ
Plat	Plasminogen activator, tissue type
Plaur	Plasminogen activator, urokinase receptor
proBDNF	Precursor of brain-derived neurotrophic factor
qPCR	Quantitative polymerase chain reaction
ROUT	Nonlinear regression and outlier removal
tPA	Tissue-type plasminogen activator
uPAR	Urokinase plasminogen activator receptor

Introduction

Learning and memory are essential cognitive functions that allow us to acquire, retain and retrieve information about the world around us, forming the basis of postnatal cognitive development [18]. The efficiency of learning and memory relies on the ability to recognise and integrate novel information [17]. Exposure to novelty enhances the subsequent learning process by promoting long-term memory formation, a phenomenon known as behavioural tagging [57]. Behavioural tagging, a process that participates in the formation of both hippocampus- and cerebral cortex-dependent long-term memories, largely relies on the synthesis of synaptic plasticity-related proteins induced by novel stimuli [3, 29]. Novelty recognition, learning and memory act within specific populations of brain neurons and involve the coordination of different molecular and cellular events, including activity-regulated gene expression in activated neurons [17, 18]. Immediate early genes are the genes whose expression is rapidly upregulated after a given cellular stimulus (e.g., in response to neuronal activity) without the requirement for de novo protein synthesis [4]. The products of immediate early genes include transcription factors (e.g., c-Fos), effector proteins acting at the synaptic level (e.g. synaptic plasticity-related proteins), non-coding RNAs and others [1, 4, 51]. Immediate early genes are implicated in learning, memory, novelty recognition, and synaptic plasticity [13, 27, 40, 56].

We have recently described that two activity-regulated genes, *Plat* (encoding tissue plasminogen activator, tPA) and *Plaur* (encoding urokinase receptor, uPAR), act as immediate early genes in response to generalized neuronal activation in seizure model [50]. Both tPA and uPAR are a part of the plasminogen activator system, which also comprises urokinase uPA and plasminogen activator inhibitors (PAI-1 and PAI-2) [16, 47]. uPA and tPA are serine proteases that catalyze the conversion of plasminogen to plasmin, a protease that can degrade fibrin and other extracellular matrix proteins [47]. In the central nervous system, tPA promotes extracellular matrix degradation, cell migration, proteolysis and activation of growth factors, neurotrophic factors (e.g. precursor of brain-derived neurotrophic factor, BDNF) and NMDA receptors [47]. tPA is, therefore, an important molecular player in synaptic plasticity, dendritic spine pruning, learning, memory formation, and fear response, but also in excitotoxicity, brain inflammation and increased brain-blood barrier permeability [15, 24, 26]. uPAR is a cellular membrane receptor for uPA that enhances the enzyme's activity, stimulates pericellular proteolysis and triggers intracellular signalling that promotes cell survival, proliferation, migration, neurite formation and branching [11, 22, 38, 39, 45, 46, 49]. Both tPA and uPAR promote neuronal migration in developing mouse brains [12, 44, 48]. *PLAUR* gene polymorphisms are associated with autism spectrum disorders [6, 7]. Gene variants of tPA- and uPA/uPAR-interacting low-density lipoprotein receptor-related protein 1 (*LRP1*) are implicated in schizophrenia risk [37, 52]. This evidence suggests that tPA and uPAR are involved in brain and neuronal functioning, and contribute to synaptic plasticity. They thus could be potential candidates for plasticity-related proteins that are produced in response to novelty exposure and participate in long-term memory formation.

This study aimed to determine whether exposure to environmental novelty induces changes in the expression of the activity-regulated immediate early genes *Plaur* and *Plat* in the mouse brain. The exploration of an open field (OF), a model of environmental novelty experience, was found to rapidly (within 1 h) induce the expression of *Plaur* and *Plat* genes in the posterior cortex and hippocampus. The upregulation of tPA was observed at the protein level, while uPAR was not detectably expressed in the brain. Exposure to an enriched environment (EE), another type of environmental novelty, also

induced early upregulation of *Plaur*, but not *Plat*, in the anterior cortex. These findings contribute to the understanding of how novelty experiences influence immediate early gene expression in the mouse brain. Investigating the modulation of plasminogen activator gene expression during exposure to novelty and learning could reveal novel molecular targets that elucidate the mechanisms underlying the neuronal encoding of cognitive information.

Materials and Methods

Animals

Adult male C57BL/6 J mice (RRID: IMSR_JAX: 000664) aged 12–14 weeks, weighing 28.2 ± 3.7 g, were obtained from the SPF-vivarium of the Center for Collective Use of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia). Mice were maintained on a standard 12-h light cycle at constant temperature (22 ± 2 °C) and humidity (45–65%). Water and food were available ad libitum. The study was carried out in accordance with the PREPARE Guideline and the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The study carefully considered the minimum number of animals required for valid results, and efforts were made to minimize pain and distress during the experiments. The conditions for keeping animals and experimental procedures were approved by the Bioethics Commission of Lomonosov Moscow State University.

Open field exposure

Open field (OF) exposure was used as a model of environmental novelty. Mice were placed in the center of the OF apparatus (44 × 44 × 44 cm with gray surface and walls) and were allowed to freely explore the arena for 10 min. The OF was cleaned with a 70% ethanol solution between animals to remove odours. Mice were then returned to their home cages. Control mice (0 h) were directly taken from their home cages. A total of 65 animals were randomly assigned to five experimental groups (0.5 h, 1 h, 3 h, 24 h, and 72 h) and one control group (0 h), each comprising 6–12 mice (biological replicates). At their respective time points after OF exposure, mice were lethally euthanized by cervical dislocation. Brains were then removed and either dissected into specific brain structures (anterior cortex, posterior cortex, and hippocampus as described previously [50, 54] or prepared for immunofluorescent staining. The experimental setup and its main stages are shown in **Supplementary Fig.** 1.

Enriched environment exposure

Enriched environment (EE) exposure was used as another type of environmental novelty. Mice (n = 60) were placed in an EE—a large rodent cage (80 × 50 × 70 cm) with lattice walls and a plastic floor. Novel objects, such as shelters, ladders, a running wheel, and tunnels, were placed in the cage. In addition, the mice were fed a novel diet containing seeds and granules. Every two days, the objects were rearranged and some were replaced with new ones to maintain novelty. Mice were kept in EE for 1 h, 3.5 h, 24 h, or 14 days. Control mice (0 h) were directly taken from their home cages. At indicated time points, mice were euthanized by cervical dislocation and the brain was removed and dissected into the anterior cortex, posterior cortex, and hippocampus. The brain tissues were frozen in liquid nitrogen and stored at −80 °C until RNA was extracted. Each experimental and control group consisted of 6–12 mice (biological replicates). The experimental setup and its main stages are shown in **Supplementary Figure** S1.

RNA isolation, reverse transcription and quantitative PCR (qPCR)

Brain tissue was quickly frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Total RNA was isolated from flash-frozen brain tissue using Trizol reagent (Invitrogen, USA, no. 15596026) according to the manufacturer's protocol. The quality and quantity of total RNA were measured using a NanoDrop1000 spectrophotometer (Thermo Fisher Scientific, USA). For further cDNA synthesis, RNA with an A260/280 ratio of at least 2.00 and an A260/230 ratio of at least 1.70 was used. cDNA synthesis was carried out with 1 µg of total RNA using the MMLV set RT (Evrogen, Russia,

No. SK021) with oligo (dT) and random (dN) primers. qPCR was performed using qPCRmix-HS SYBR (Evrogen, Russia, No. PK147L) on a CFX96 Touch real-time PCR device (Bio-Rad, USA). Mouse cDNA primers were synthesized by Evrogen, Russia (**Supplementary Table** S1). The amplification program consisted of a 5-min denaturation step at 95 °C, followed by 40 amplification cycles consisting of 15 s of denaturation at 95 °C, 30 s of annealing at 62 °C, and 20 s of elongation at 72 °C. PCR for each sample was performed in three technical replicates. Relative transcript levels were calculated using the $2^{-\Delta\Delta Ct}$ method with *Actb* as the reference gene; normalization was performed by taking the average level of each transcript in the control (0 h) as one.

Immunofluorescent staining, microscopy and image analysis

For immunofluorescence staining, isolated and mouse brain samples were fixed in 4% paraformaldehyde (Panreac), washed in phosphate-buffered saline (PBS), and frozen in liquid nitrogen to prepare 5-µm-thick cryostat sections. Sections were then dried, washed, and blocked with 10% secondary antibody donor serum supplemented with 1% bovine serum albumin (BSA, Sigma-Aldrich) for 1 h. After washing in PBS, sections were incubated overnight with primary antibodies against uPAR (Abcam, cat. #Ab103791), tPA (Cloud-Clone, cat. #PAA525Mu01), and neurofilament-200 (NF200, neuronal marker, MyBioSource, cat. #MBS175078) or non-immune IgG as a control for staining specificity. Samples were then washed three times with PBS and incubated with secondary antibodies conjugated to Alexa Fluor®594 and Alexa Fluor®488 (1:1000 dilution, Molecular Probes) for 1 h at room temperature. To visualize nuclei, samples were counterstained with DAPI (Sigma Aldrich). Samples were washed with PBS and mounted in an Aqua Poly Mount polymerisation medium (Polysciences). Images were acquired using a Leica DFC 7000 fluorescent microscopy with a 10× and 20× objective. DAPI, AlexaFluor®488, and AlexaFluor®594 fluorescences were sequentially excited using lasers with 405, 488, and 594 wave lengths, respectively. All images were captured with the same confocal gain and offset settings. The specificity of the staining was confirmed using non-immune IgG antibodies (**Supplementary figure** S2).
To quantify tPA protein expression in brain tissue, images were processed using Fiji software [42] (ImageJ version 2.1.0/1.53c). The boundaries of visible brain tissue were manually delineated using the polygon selection tool, the mean grey value in the AF488 (green) channel was measured and normalized to the mean values in control (0 h) samples.

Statistical analysis

All data were analyzed using GraphPad Prism 9 software (GraphPad Software Inc., USA) and represented as individual values, mean ± SEM. Data were tested for normality of distribution using the D'Agostino-Pearson normality test. Outliers were excluded using the nonlinear regression and outlier removal (ROUT) method with Q = 1%, and data were re-checked for normality. Unpaired t-test was used to compare the data between two groups. ANOVA followed by Dunnett's post hoc test was applied for the comparison of experimental groups versus the control group (0 h). The unit of analysis was one animal. Values of $p < 0.05$ were considered statistically significant.

Results

The primary objective of the present study was to investigate whether the novelty experience alters the expression of activity-regulated immediate early genes *Plaur* and *Plat* in mouse brain tissue. Beyond its classical application in neurobehavioral studies, OF exploration was previously described as a model of novelty experience in mice [19, 58]. We, therefore, examined whether brief exposure to OF (10 min) resulted in short- and long-term changes in gene expression in mouse brains at time points ranging from 30 min to 3 days after OF exposure.
To validate the model, we first evaluated the expression of *Arc*, an established immediate early gene that was previously shown to be rapidly upregulated in neurons after exposure to a novel environment [14, 56]. As expected, the exposure to OF led to a prominent increase in *Arc* expression in the anterior cortex (9.728 ± 2.321 vs 1.000 ± 0.124 at 0 h, $p = 0.0005$), in the posterior cortex (5.977 ± 0.991 vs 1.000 ± 0.229 at 0 h, $p = 0.0094$) and hippocampus (24.00 ± 2.176 vs 1.000 ± 0.142, $p < 0.0001$) (Fig. 1B, 1C) within 1 h. Futher analysis showed that exposure to environmental novelty resulted in a rapid significant induction of *Plaur* expression in the posterior cortex (3.362 ± 0.335 vs 1.000 ± 0.138 at 0 h, $p < 0.0001$) and hippocampus (1.729 ± 0.207 vs 1.000 ± 0.125, $p = 0.0303$) already at 1 h after OF exploration (Fig. 1E, 1F). At 24 h after

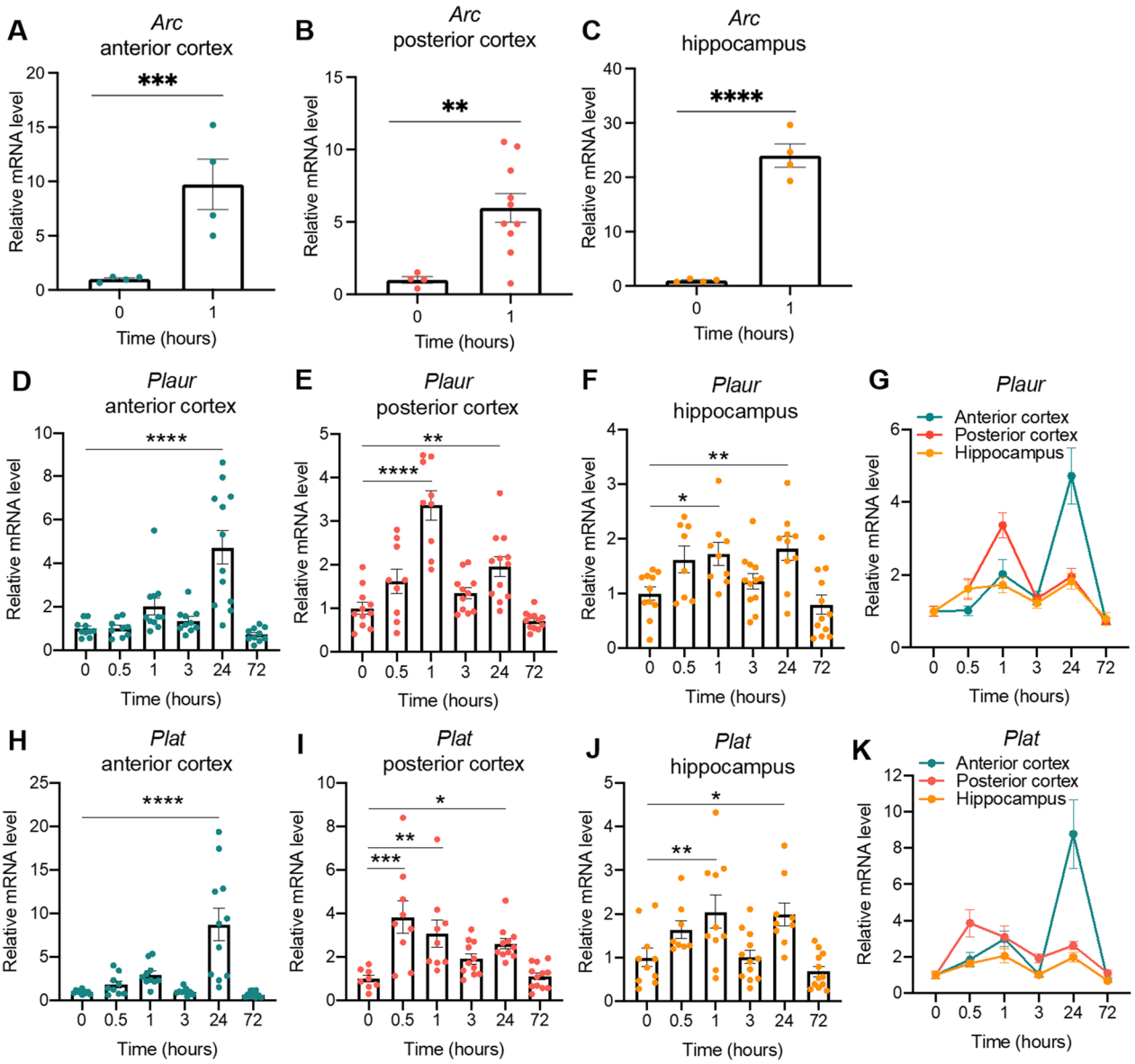

Fig. 1 The level of *Arc*, *Plaur* and *Plat* relative expression in mouse brain after open field environmental novelty: (A, D, H) in the anterior cortex; (B, E, I) in the posterior cortex; (C, F, J) in the hippocampus and (F, J) in three regions combined. (A-C) upaired t-test, (D-K) ANOVA, Dunnett's post hoc test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. Data are shown as individual values, mean ± SEM. Each time point group comprised 9–12 mice

OF exploration, a significant increase in *Plaur* expression was observed in all brain regions examined: in the anterior cortex (4.724 ± 0.769 vs 1.000 ± 0.116 at 0 h, $p<0.0001$), posterior cortex (1.961 ± 0.222 vs 1.000 ± 0.138 at 0 h, $p=0.0039$), and hippocampus (1.828 ± 0.211 vs 1.000 ± 0.125, $p=0.0084$) (Fig. 1D-1F). The most potent *Plaur* induction at 1 h was observed in the posterior cortex, while at 24 h—in the anterior cortex (Fig. 1G). At 72 h after OF exposure, *Plaur* expression returned to basal levels in all brain regions examined (Fig. 1D-1G).

Similar to *Plaur*, *Plat* expression was also rapidly and significantly induced in the mouse brain by exposure to OF. Already at 0.5 h after OF exploration, *Plat* was significantly upregulated in the posterior cortex (3.851 ± 0.751 vs 1.000 ± 0.162 at 0 h, $p=0.0001$, Fig. 1I). At 1 h after OF exploration, *Plat* expression was significantly increased in both the posterior cortex (3.092 ± 0.629 vs 1.000 ± 0.162 at 0 h, $p=0.0053$) and hippocampus (2.053 ± 0.379 vs 1.000 ± 0.211 at 0 h, $p=0.0094$) (Fig. 1I, 1J). At 24 h after OF exploration, *Plat* expression was significantly elevated in all brain regions examined: in the anterior cortex

(8.772 ± 1.880 vs 1.000 ± 0.072 at 0 h, $p < 0.0001$), the posterior cortex (2.627 ± 0.233 vs 1.000 ± 0.162 at 0 h, $p = 0.0301$) and hippocampus (1.988 ± 0.264 vs 1.000 ± 0.211 at 0 h, $p = 0.0202$) (Fig. 1H-1J). At 1 h, the posterior cortex showed the strongest *Plat* induction, while at 24 h, the strongest *Plat* induction was observed in the anterior cortex (Fig. 1K). In all studied brain regions, 72 h after OF exposure, *Plat* expression went back to its baseline levels (Fig. 1H-1K).

In addition to *Plaur* and *Plat*, we also examined the expression of *Plau* (encodes for uPA) and *Serpine1* (encodes for PAI-1) genes, two other components of the plasminogen activator system. In contrast to the results obtained in the generalized seizure model [50], where both *Plau* and *Serpine1* responded lately to neuronal activation (72 h after seizure induction), we observed an early, although not uniform, upregulation of *Plau* and *Serpine1* genes in response to OF exposure. In the hippocampus, *Plau* was upregulated at 1 h after OF exposure (6.421 ± 2.201 vs 1.000 ± 0.171 at 0 h, $p = 0.0027$, **Supplementary figure** S3C). In the anterior cortex, *Plau* was upregulated at 3 h after OF exposure (4.369 ± 1.305 vs 1.000 ± 0.150 at 0 h, $p = 0.0008$, **Supplementary figure** S3A). In the posterior cortex, *Plau* was upregulated at 24 h after OF exposure (103.734 ± 57.982 vs 1.000 ± 0.428 at 0 h, $p = 0.0010$, **Supplementary figure** S3B). An early upregulation was also observed for *Serpine1*. In the anterior cortex, *Serpine1* was upregulated at 1 h after OF exposure (4.369 ± 1.305 vs 1.000 ± 0.127 at 0 h, $p = 0.0196$) and at 24 h after OF exposure (4.237 ± 0.843 vs 1.000 ± 0.127 at 0 h, $p < 0.0001$) (**Supplementary figure** S4A). In the posterior cortex, *Serpine1* was upregulated at 0.5 h after OF exposure (1.946 ± 0.194 vs 1.000 ± 0.121 at 0 h, $p = 0.0041$) and at 1 h after OF exposure (2.414 ± 0.318 vs 1.000 ± 0.121 at 0 h, $p < 0.0001$) (**Supplementary figure** S4B). In the hippocampus, *Serpine1* was upregulated at 1 h after OF exposure (2.511 ± 0.676 vs 1.000 ± 0.142 at 0 h, $p = 0.0065$, **Supplementary figure** S4C).

Next, to investigate whether changes in protein expression follow changes in mRNA expression, we analyzed the expression of uPAR and tPA in the cortex and hippocampus after OF exposure. The results of double immunofluorescence staining of the cortex and hippocampus with antibodies to uPAR and NF200 (neuronal marker) are presented in Fig. 2. In the cortex and hippocampus, uPAR expression was low to undetectable in both control (0 h) and 4 h after OF exposure (Fig. 2A-B). tPA expression was also undetectable in the cortex (Fig. 3A). In contrast, tPA was abundantly expressed in the hippocampus of mice in control conditions (Fig. 3B). Partial colocalization of tPA and NF200 signals was observed, providing evidence for neuronal expression of tPA. In the hippocampus tPA expression was also present after 4 h and was significantly upregulated at 24 h after OF exposure (1.159 ± 0.0841 at 4 h and 1.748 ± 0.210 at 24 h vs 1.000 ± 0.0860 at 0 h, $p = 0.5904$ for 4 h and $p = 0.0057$ for 24 h, Fig. 3B, 3C). In the cortex, tPA expression was undetectable (Fig. 3C, 3D). Overall, these results confirm our previous findings that *Plat* was upregulated in response to OF exposure in hippocampus.

Exposure to an EE was also previously employed as a model of environmental novelty experience in mice [19]. We next decided to investigate whether EE exposure also induces changes in the expression of *Arc*, *Plaur* and *Plat* genes. In contrast to the relatively brief and acute novelty experience in OF, this time, mice were placed for longer periods in a more complex and constantly changing environment. We found that presence in an EE led to an early upregulation of *Arc* in the posterior cortex of mice at 1 h (4.199 ± 0.609 vs 1.000 ± 0.130 at 0 h, $p = 0.0039$) and in hippocampus (3.982 ± 0.486 vs 1.000 ± 0.191 at 0 h, $p = 0.0004$) (Fig. 4B, 4C). No significant differences in *Arc* expression were observed in the anterior cortex (Fig. 4A). *Plaur* expression was induced in the anterior cortex of mice, at 1 h (1.705 ± 0.138 vs 1.000 ± 0.188 at 0 h, $p = 0.0032$) and 3.5 h (1.922 ± 0.180 vs 1.000 ± 0.188 at 0 h, $p = 0.0001$) (Fig. 4D). 24 h after being in EE, *Plaur* expression returned to the level of control mice and remained unchanged after 14 days of EE (Fig. 4D, 4G). There was a similar tendency for early *Plaur* upregulation in the hippocampus after EE exposure, but it did not reach statistical significance (1.734 ± 0.344 at 1 h and 1.733 ± 0.249 at 3.5 h vs 1.000 ± 0.136 at 0 h, $p = 0.0765$ and $p = 0.0769$ respectively, Fig. 4F). No significant differences in *Plaur* expression after EE exposure were observed in the posterior cortex (Fig. 4E). *Plat* expression in the anterior cortex and hippocampus was not significantly changed by EE (Fig. 4H, 4J). However, *Plat* expression was significantly downregulated in the posterior cortex 24 h after being in EE (0.384 ± 0.025 vs 1.000 ± 0.165 at 0 h, $p = 0.0006$, Fig. 4I). *Plat* expression returned to the level of control values 14 days after being in EE (Fig. 4I, 4K).

Discussion

The primary objective of the present study was to investigate whether the experience of novelty is accompanied by a change in the expression of activity-regulated immediate early genes, *Plaur* and *Plat*, in brain tissue. The study design incorporated two distinct paradigms of environmental novelty exposure: OF exploration and EE. The former, a brief exposure to a novel environment, and the latter, an extended exposure to a complex, constantly changing and stimulating

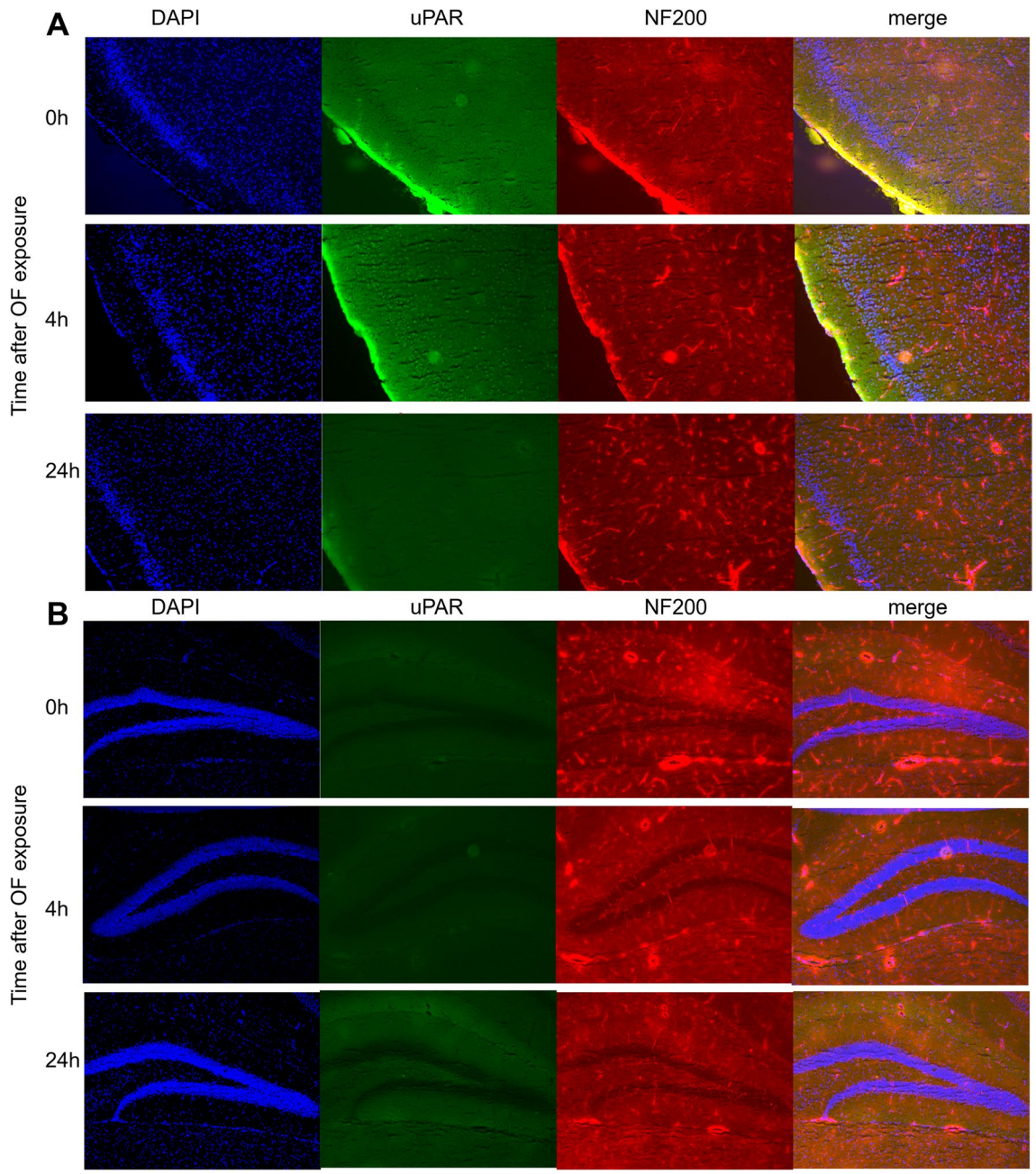

Fig. 2 Immunofluorescent staining of uPAR and NF200 in the mouse cortex and hippocampus at 0 h, 4 h, and 24 h after open field (OF) exposure. Representative images of the cortex (A) and hippocampus (B) are shown. Mouse brain tissue was stained with antibodies against uPAR (green) and neurofilament 200 (NF200, red). Nuclei were counterstained with DAPI (blue)

environment, allowed for the examination of gene expression patterns in response to varying degrees of cognitive demand. We and others have previously shown that both *Plat* [34] and *Plaur* [50] genes are rapidly upregulated in response to generalized neuronal activation (pentylenetetrazole-induced seizures), but whether their expression is regulated by other stimuli in the brain remains unclear. Our results here showed that short-term exposure to an OF resulted in rapid and significant induction of *Plaur* and *Plat* gene expression in the hippocampus and posterior cortex of mouse brains (Fig. 1). Immunofluorescence staining confirmed elevation of tPA protein levels in response to novelty: tPA content in mouse brains was significantly increased at 24 h following OF exposure compared to controls (Fig. 3). Furthermore, *Plaur*, but

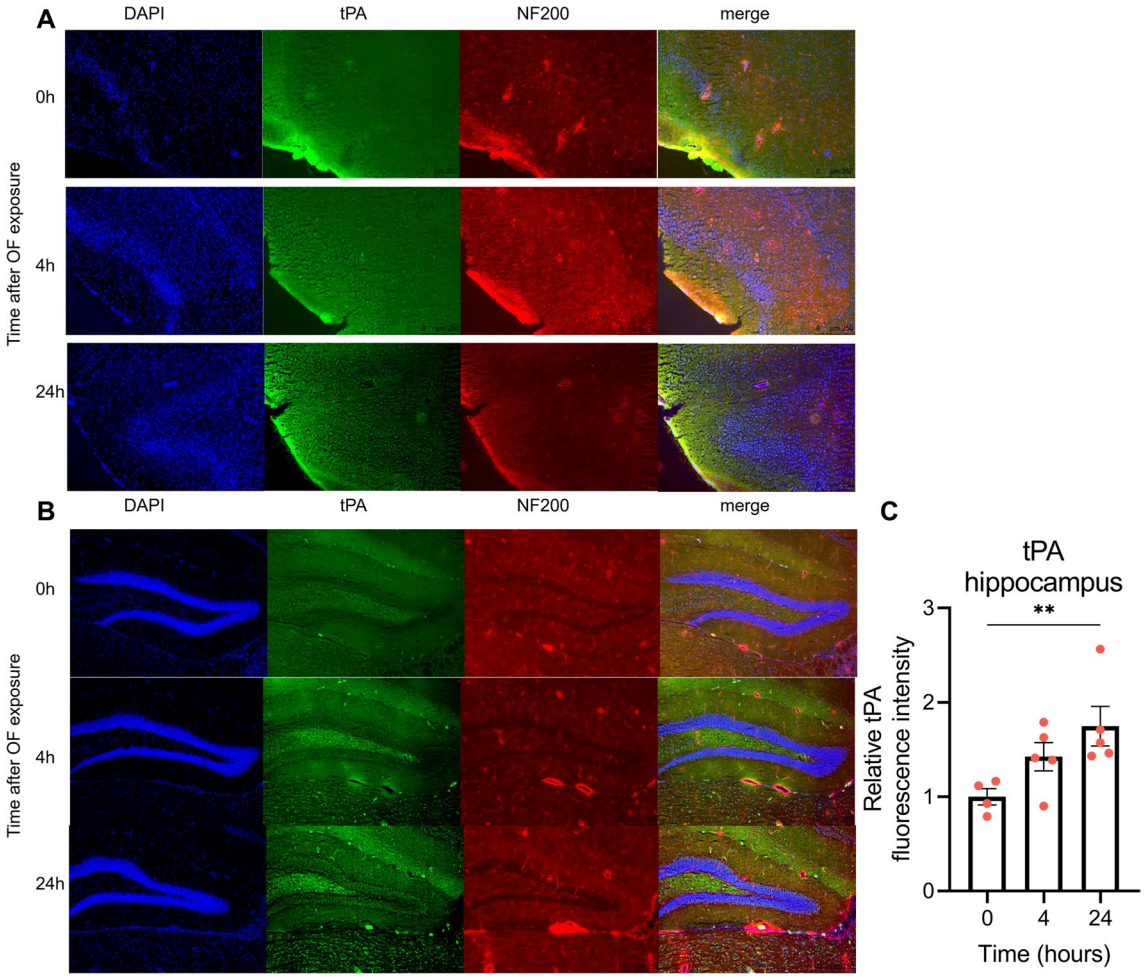

Fig. 3 Immunofluorescent staining of tPA and NF200 in the mouse cortex and hippocampus at 0 h, 4 h, and 24 h after open field (OF) exposure. (A, B) Representative images of the cortex за (A) and hippocampus (B) are shown. Mouse brain tissue was stained with antibodies against tPA (green) and neurofilament 200 (NF200, red). Nuclei were counterstained with DAPI (blue). (C) The mean relative fluorescence intensity of tPA in the hippocampus. ANOVA, Dunnett's post hoc test, ** $p < 0.01$. Data are shown as individual values, mean ± SEM. Each time point group comprised 4–6 mice

not *Plat*, was early upregulated after more complex and prolonged environmental novelty—presence in the EE (Fig. 4). The observed deregulations of *Plaur* and *Plat* gene expression in the brain suggest their involvement in the molecular cascades triggered by novel stimuli.

Exposure to novelty is believed to induce the synthesis of plasticity-related proteins, inducing local (and potentially transient) molecular changes at synapses, which could transform transient forms of memory into long-lasting ones, a concept of behavioural tagging [32]. The contribution of immediate early genes in novelty processing, long-term potentiation and memory consolidation has been previously documented. For instance, the immediate early genes *Arc* and *Homer1a* are upregulated in neurons in the hippocampus and neocortex after exposure to a novel environment [56] and have been implicated in synaptic plasticity, learning and memory consolidation [8, 28]. Immediate early genes are transiently induced in response to stimulation, and *Plaur* and *Plat* genes exemplify this pattern, exhibiting a return to baseline expression levels within three hours after OF exposure (Fig. 1). The early induction of *Plaur* and *Plat* genes suggests their involvement in rapid memory acquisition processes associated with novelty exposure and implies that may play important roles in the novelty-dependent modulation of synapses in order to encode memory. A second peak in their expression is observed after 24 h of OF exploration aligns with the slower, multi-stage process of memory formation, encompassing memory consolidation and neural network restructuring. This two-wave (rapid and delayed) induction pattern after OF exploration has also been observed for *Arc* [36]. The differential induction of *Plaur* and *Plat* expression in response to

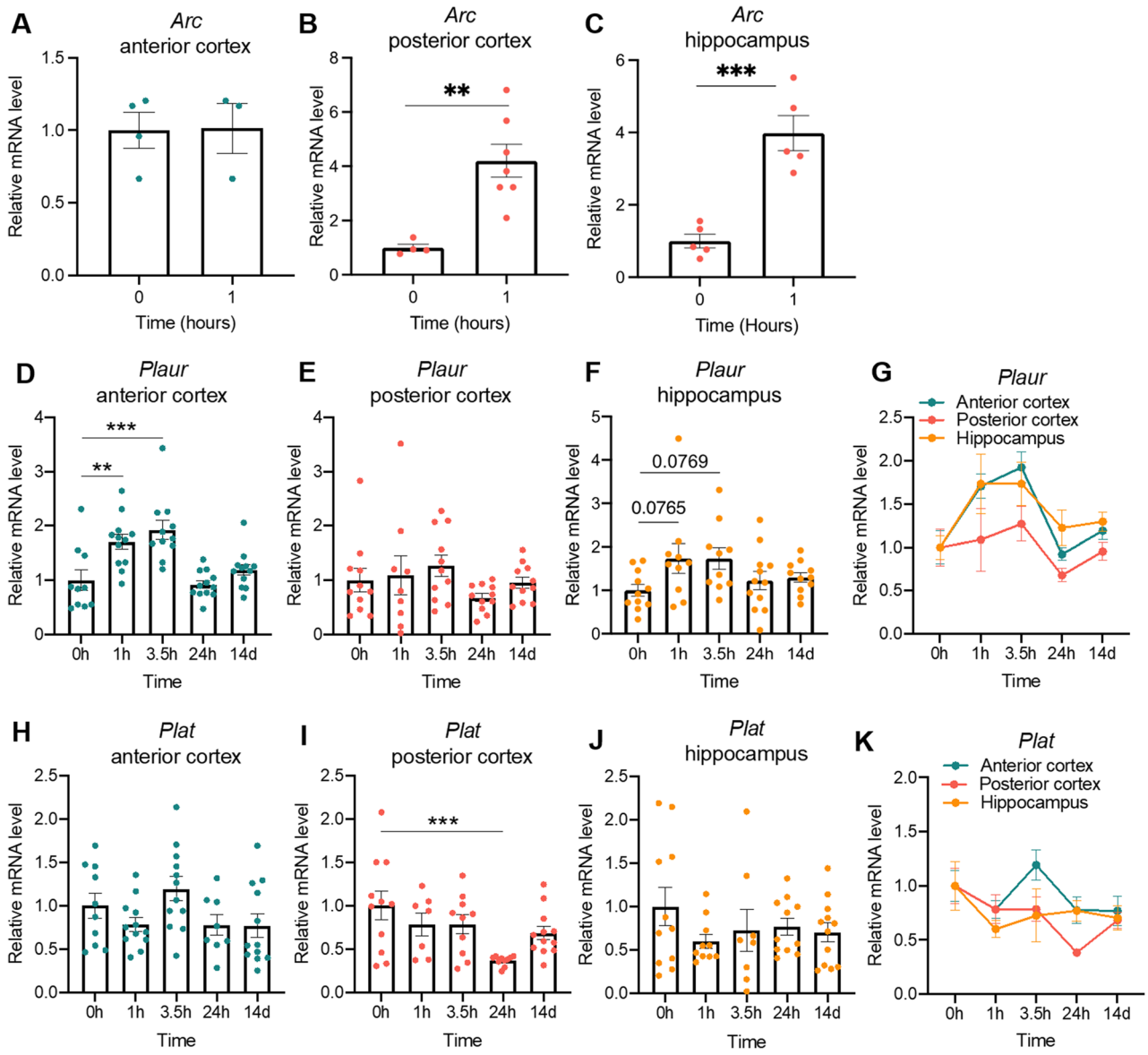

Fig. 4 The level of *Arc*, *Plaur* and *Plat* relative expression in mouse brain after exposure to enriched environment novelty: (A, D, H) in the anterior cortex; (B, E, I) in the posterior cortex; (C, F, J) in the hippocampus and (G, K) in three regions combined. (A-C) upaired t-test, (D-K) ANOVA, Dunnett's post hoc test, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Data are shown as individual values, mean ± SEM. Each time point group comprised 7–12 mice

OF and EE exposure suggests that these genes may be differentially regulated by different types of novelty experiences. OF exposure, which involves more intense and immediate exposure to novelty, induced the expression of both *Plaur* and *Plat*. In contrast, EE exposure, which provides a more gradual and sustained exposure to novelty, induced the expression of *Plaur* only in the anterior cortex. The differential expression in response to OF and EE environmental novelties was described for a plasticity-related protein kinase Mζ (PKMζ) [19].

tPA is normally expressed in the brain parenchyma by neurons and glial cells and its expression is rapidly upregulated in an activity-dependent manner [15, 41, 55], which is in line with our findings (Fig. 3). tPA may be involved in regulating synaptic plasticity through various mechanisms, including extracellular matrix degradation, cleavage and maturation of the neurotrophic factor proBDNF into BDNF, cleavage of neuronal cell adhesion molecule (NCAM), activation of NMDA receptors, activation of the extracellular regulated kinase (ERK)1/2 signal transduction and regulation of neuronal migration

and outgrowth [5, 15, 25, 30, 31]. ERK1/2 pathway is one of the key cellular pathways involved in the synthesis of plasticity-rMadani Solbergelated proteins upon memory consolidation [35]. tPA knockout in mice impairs the learning process [43], while tPA overexpression improves mice performance in learning tasks [23], suggesting its important role in information processing.

Under physiological conditions, uPAR protein expression in the adult mouse brain is low to undetectable [9, 53], which is consistent with our findings (Fig. 2). However, uPAR expression can be significantly induced in response to various stimuli, such as injury, inflammation, seizures, or in different pathologies, such as Alzheimer's disease, Creutzfeld-Jakob disease, and multiple sclerosis [2, 33, 50]. Early *Plaur* mRNA upregulation was robustly seen in response to both OF and EE novelty (Fig. 1, 4), uPAR protein expression, however, was not detected until 24 h after OF exposure and was limited to several signals in the field of view. This dynamic relationship suggests a nuanced regulatory mechanism governing *Plaur* mRNA translation. Notably, spatially restricted local mRNA translation (protein synthesis) is an important mechanism for the regulation of neuronal functioning, including synaptogenesis, synaptic signal transmission and plasticity [59]. Whether *Plaur* mRNA is a subject of spatial translational repression and stimulus-induced translational activation requires further exploration. uPA/uPAR-dependent signalling promotes synaptic recovery in the ischemic brain [10] and the role of this system in learning and memory consolidation in physiologic conditions remains to be explored. Single nucleotide polymorphisms in the *PLAUR* gene are associated with an increased risk of autism spectrum disorder [6]. *Plaur* knockout in mice disrupts their social interaction [21], while double *Plau*/*Plaur* knockout mice are more social with other mice and show less interest in their surrounding environment [20]. Whether these observations reflect solely developmental defects in the brain or implicate the uPA/uPAR system involvement in adult brain function and information processing, or both, remains to be elucidated. Our data presented here for the first time suggest that uPAR might be a part of the brain's physiological response to novel information processing.

To conclude, we found that *Plaur* and *Plat* are rapidly upregulated in the mouse cortex and hippocampus in response to environmental novelty experiences. This suggests that they may play a role in the synaptic plasticity during novelty recognition, learning and memory. Further study of the functions of *Plaur* and *Plat* will strengthen our understanding of how novel information is processed in the brain.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical statement The study was carried out in accordance with the PREPARE Guideline and the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The study carefully considered the minimum number of animals required for valid results, and efforts were made to minimize pain and distress during the experiments. The conditions for keeping animals and experimental procedures were approved by the Bioethics Commission of Lomonosov Moscow State University.

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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